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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This is a pre-clinical study to establish the effectiveness anti-inflammatory approaches on improving recovery from traumatic brain injury. The studies employ rats and pigs, and use blast injury and controlled cortical injury (CCI) models. We aim to transiently suppress inflammation with veliparib (an inhibitor of poly(ADP-ribose) polymerase which thereby suppresses NF-kB – mediated inflammatory responses), intranasal NAD, (a natural metabolite which we have in prior studies shown to also suppress poly(ADP-ribose) polymerase activity and inflammatory responses) and ketogenic diet. CtBP1/2 knockout mice will be generated to test a specific mechanisms by which ketogenic diet can have anti-inflammatory effects. For all studies, outcome measures include histological indices of inflammation, cell death, and axonal injury, with behavioral indices of motor coordination, cognitive function, and anxiety, and with electrocorticography measures of brain network activity. Studies accomplished in year one have established dose-effectiveness for veliparib, have characterized the physical features and histological effects of rat and pig CCI and blast models, and have established quantifiable indices of motor and cognitive recovery. These studies have also shown that electrocorticography can reliably track changes in brain network activity as motor function recovers.

15. SUBJECT TERMS

brain injury, blast injury, rat, pig, inflammation, metabolism, microglia

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1. INTRODUCTION

This is a pre-clinical study to establish the effectiveness of anti-inflammatory approaches on improving recovery from traumatic brain injury. The studies employ rats and pigs, and use blast injury and controlled cortical injury models. We suppress inflammation in one of three ways: (1) a commercially available, FDA –approved drug, veliparib given for 7 days beginning 24 hours after injury. Veliparib is an inhibitor of poly(ADP-ribose) polymerase and thereby suppresses NF-kB – mediated inflammatory responses. (2) Intranasal NAD, given twice at 1 and 2 hours after injury. NAD (nicotinamide adenine diphosphate) is a natural metabolite which we have in prior studies shown to also suppress poly(ADP-ribose polymerase activity and inflammatory responses. (3) Ketogenic diet, begun 12 hours after TBI. CtBP1/2 knockout mice will be generated to test a specific mechanisms by which ketogenic diet can have anti-inflammatory effects. For all studies, outcome measures include histological indices of inflammation, cell death, and axonal injury, with behavioral indices of motor coordination, cognitive function, and anxiety. An additional outcome measure will be electrocorticography, as a means of evaluating these interventions on the brain network activity that underlie recovery after injury.

2. KEYWORDS

brain injury, blast injury, mouse, rat, pig, electrocorticography, inflammation, metabolism, microglia,

3. ACCOMPLISHMENTS

What were the major goals of the project?

From the SOW: The year-1 milestones are as follows:

a) Establish blast injury models for rats and pigs
Timeline: 0- 6 months.

b) Initiate blast injury studies in rats and pigs
Timeline: 0-10 months.

c) In rats, using the controlled cortical impact (CCI) approach, establish the 'time window of opportunity' for treatment with intranasal NAD and with a PARP inhibitor (veliparib).
Timeline: 0-10 months.

d) In pigs, using the controlled cortical impact (CCI), establish the 'time window of opportunity for treatment with a PARP inhibitor.
Timeline: 0-10 months.

What was accomplished under these goals?

a & b) Establish blast injury models for rats and pigs and initiate the blast injury studies

i. Rats. A custom-made blast tube device was obtained from L3/Jaycor, installed, and characterized. The device outfitted with a diffuser creates a 100 psi overpressure peak, with negligible follow-on wind. Initial studies identified extensive inflammatory response, axonal injury, and scattered neuronal cell death in rats loosely positioned orthogonal to the blast tube.

However, we noticed that the rat heads were often rotated 30-45 degrees after blast, raising the possibility that the observed injury was due to head rotation rather than blast wave interaction with brain itself. Accordingly, we reconfigured the rodent restraint system to completely immobilize the head, and in addition placed the diffuser component to mitigate post-blast airflow against the head. Under these conditions injury was much attenuated, but still present. (see 3 panels of results below, as presented at the Neurotrauma Symposium). We are now proceeding with blast injury of fully trained rats to determine if this relatively small injury causes detectable and significant motor impairment. If it does not, we will conclude that the original aim of studying blast injury as a distinct form of brain injury, occurring independent of translational/rotational skull and brain movement induced by blast, is not tenable, and we will instead allow limited and measured movement of the head in order to evaluate a more real-life closed head injury resulting from blast exposure. To this end, we have established an arrangement with X2Bio so that we can use their patented remote sensor accelerometer to quantify head movement, if we need to go this route. Our current system also currently includes real-time blood-pressure monitoring and video-monitoring during blast.

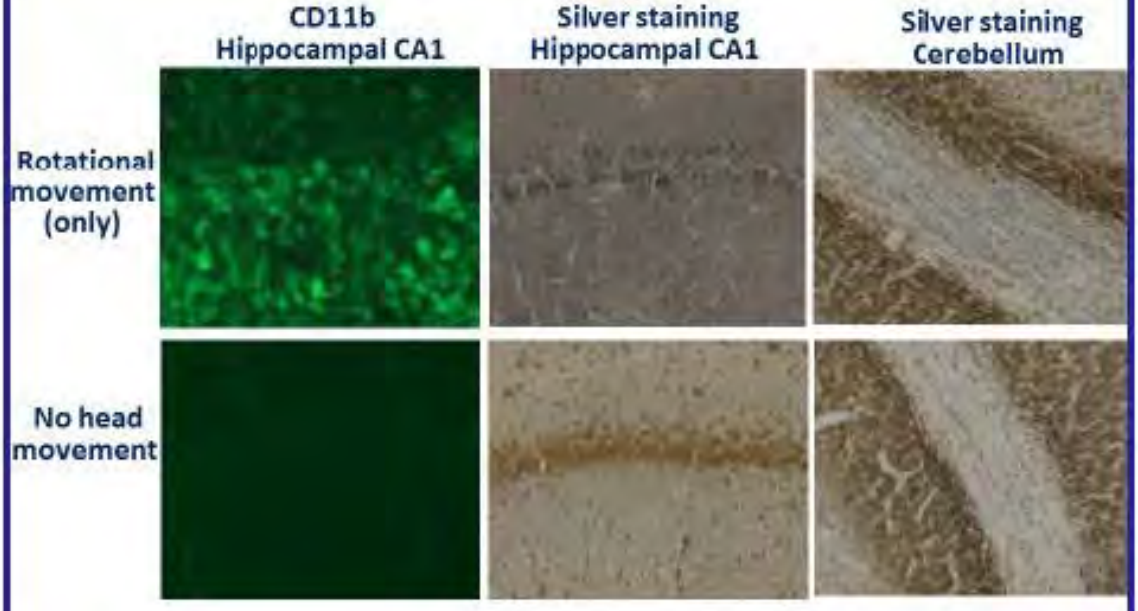
Blast Tube setup (L-3/Jaycor)

Peak pressure = 220 psi pressure
Positive pressure duration = 2.6 msec

Rotational movement (only)

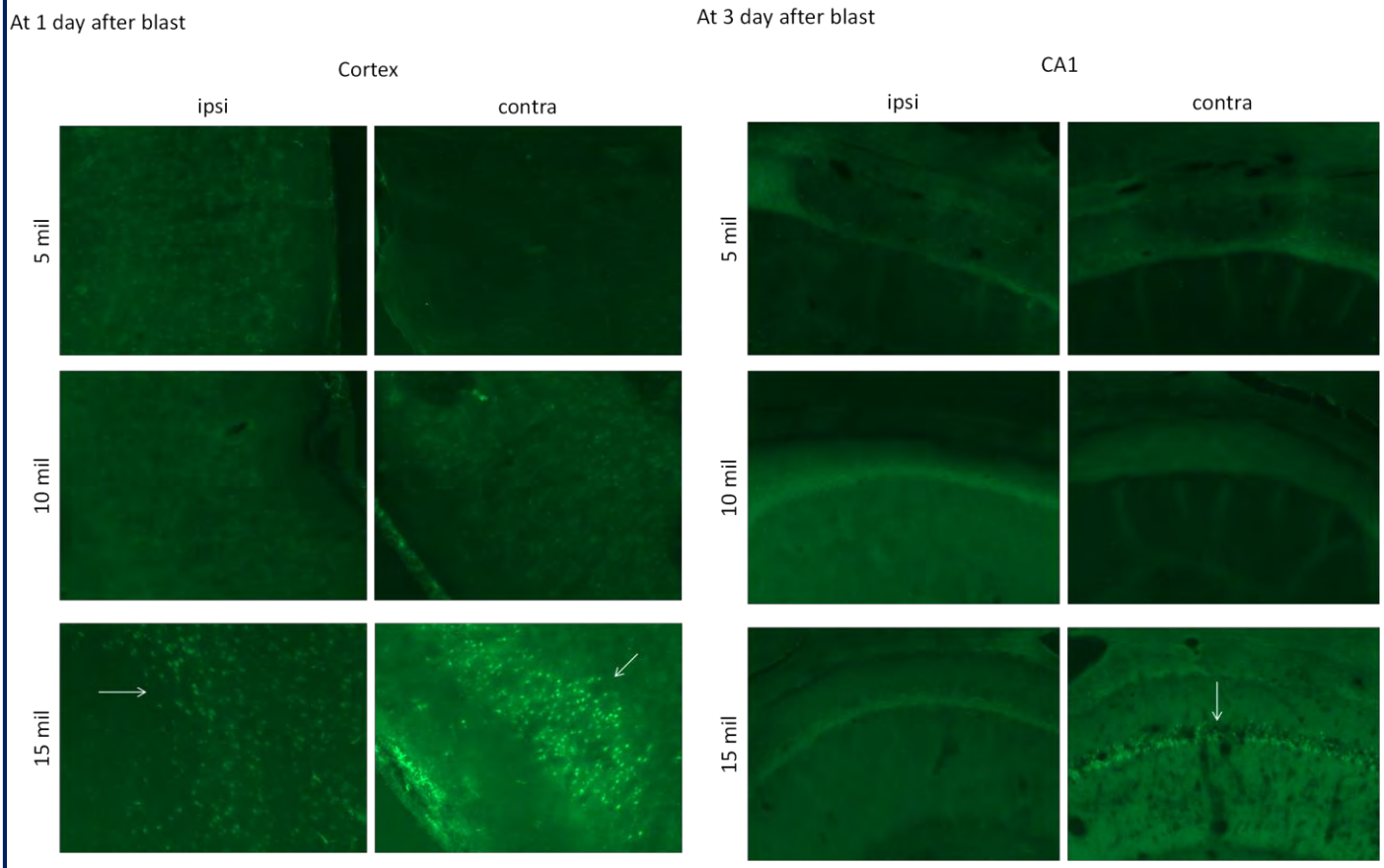
No head movement

Results

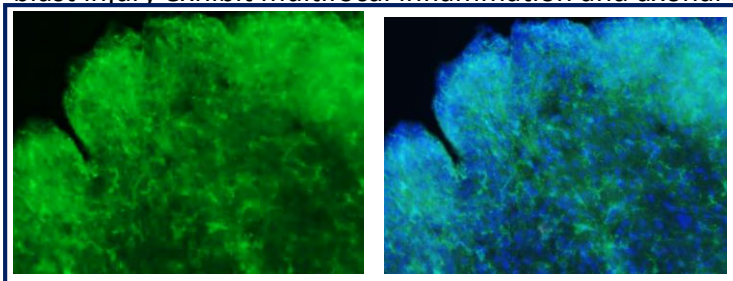


Conclusions: True blast injury is likely minimal relative to the injury caused by head / brain movement in any real-world TBI setting.

Examples of neuronal injury induced by 3 different grades of blast injury in cortex (left) and hippocampus (right). The highest grade, 15 mil, corresponds to 200 psi overpressure. Dead neurons are stained bright green by fluoro-jade B



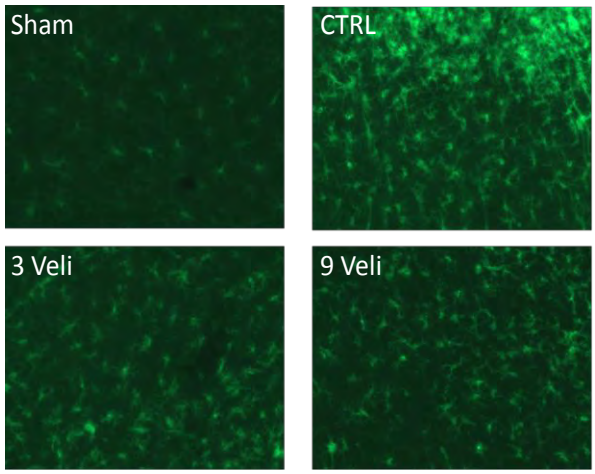
i. Pigs. A blast tube already in place in the Panter lab was characterized by L3/Jaycor as part of the subcontract. In contrast to the rat tube, the pig tube exposes the pug heads to a significant amount of bulk air movement following the blast, and the resulting translational head movement is almost certainly the primary source of brain injury resulting from this exposure. This can be seen as complementary to the true blast injury we are generating in the rat brains. Within the next two weeks at least one pig will undergo blast while outfitted with X2 accelerometers, so that the head movement can be accurately quantified. Pigs exposed to the blast injury exhibit multifocal inflammation and axonal disruption, as shown below.



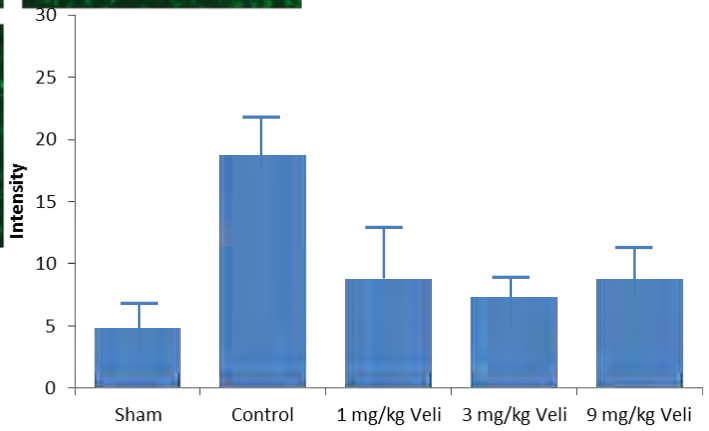
Gyrirform pig cortex after blast injury.
Green, avtivated microglia (CD11b); Blue, neuronal soma (NeuN)

c&d) <u>In rats and pigs,</u> <u>using the controlled cortical impact (CCI) approach, establish the 'time window of opportunity' for treatment with intranasal NAD and with a PARP inhibitor (veliparib).</u>

As we initiated these studies we realized that it would be necessary to first establish effective does of veliparib and NAD before establishing the maximal time window of opportunity. Moreover, "effective" must encompass both short term suppression of inflammation and long-term behavioral effects. This necessitated reordering the studies, to first establish dose-response effect on acute inflammation, then establish if this dose effects long term behavioral outcomes in each model, and then last determine the latest time after injury the drug can be given to achieve these outcomes. Accordingly, we have first established the dose-response curve for veliparib suppression of CCI-induced microglial activation, as shown below. (n = 4 in ach condition, harvested 4 days after injury)



Activated microglia shown by CD11b (green)
(CTRL = injury but no drug)

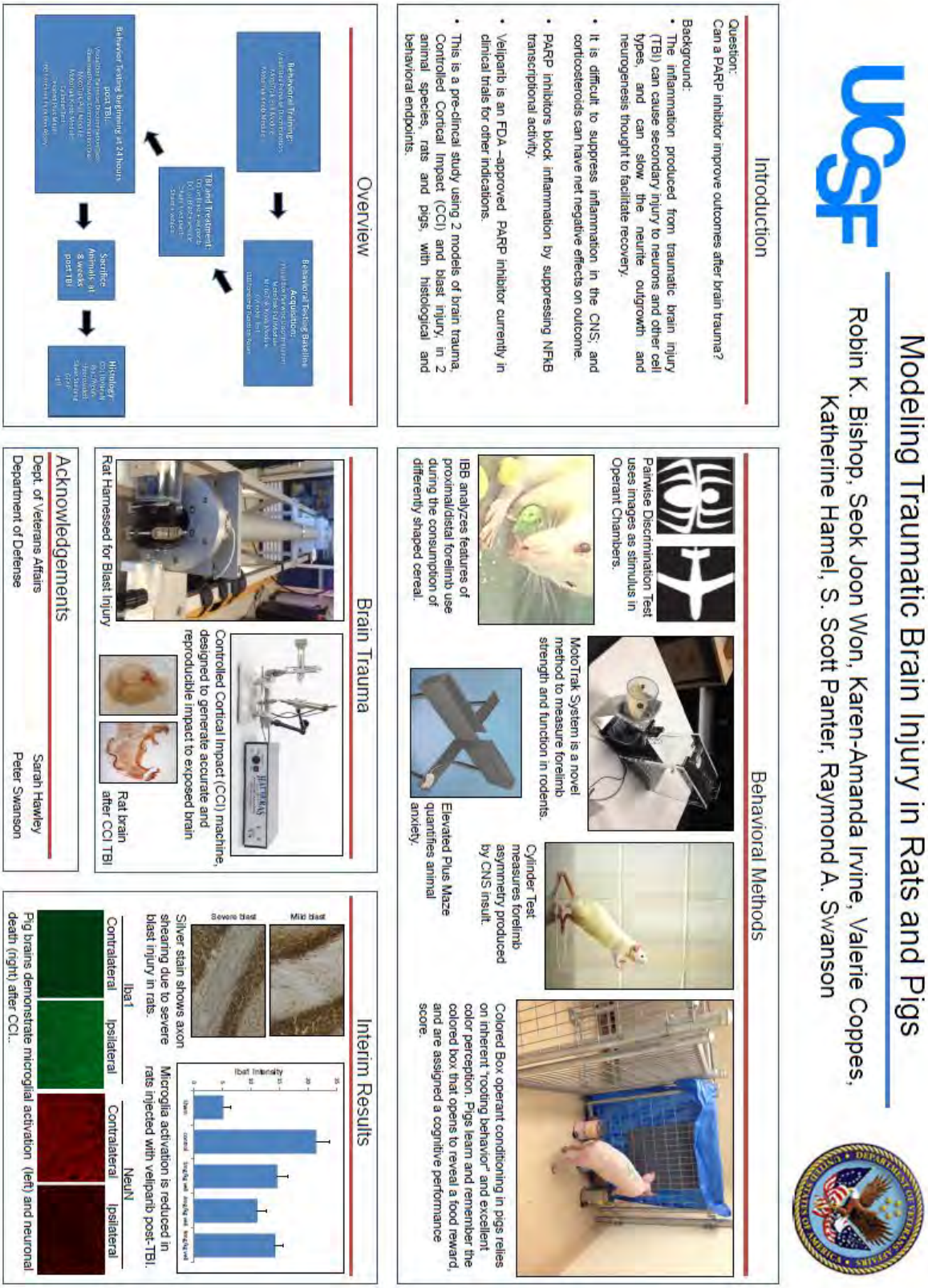


We will be supplementing these data with measures of pro-inflammatory gene expression in microglia isolated from the peri-lesional region region in the next few weeks, but in the interim we will be proceeding with 3 mg/kg as the dose of veliparib to be used in the rat studies. A parallel study has been completed in pigs (n = 4 at each dose), but those data have not yet been analyzed.

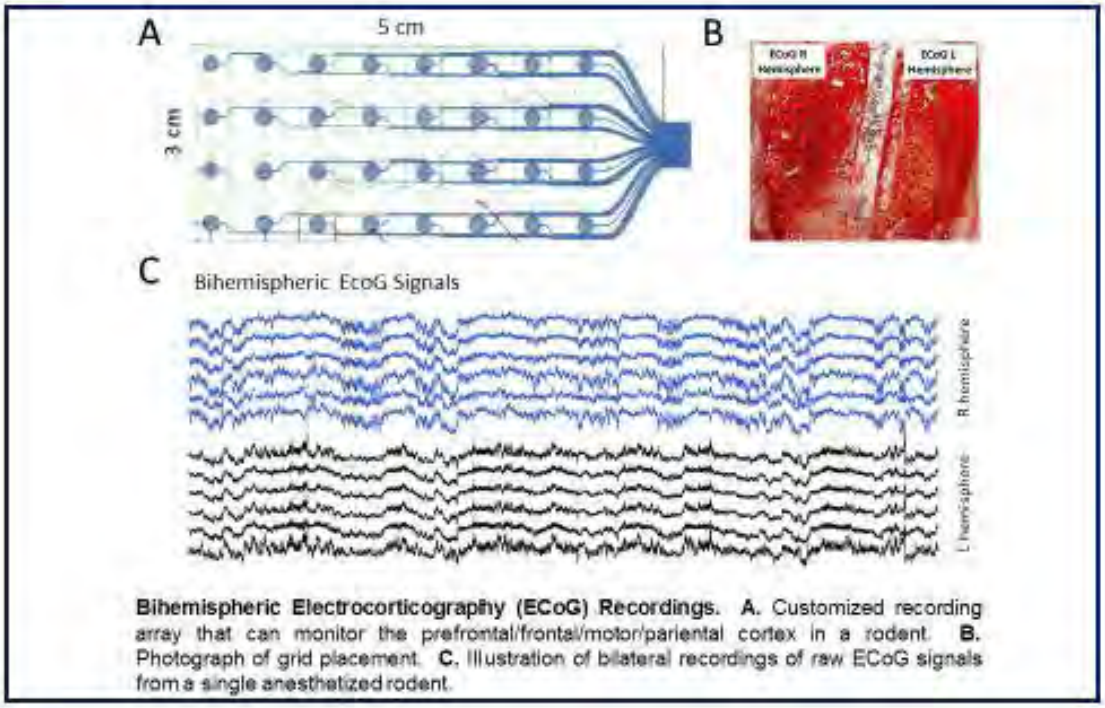
<u>Other accomplishments</u>

a) Behavioral assessments. Results of this project hinge critically on robust, quantifiable, and reproducible behavioral outcomes. To this end we have made a considerable investment in time and equipment to build an infrastructure capable of performing these measurements at the sale required for these studies. These include 2 novel and completely objective, quantifiable measures; the Mototrac measure of forelimb dexterity, and a Videobox touch

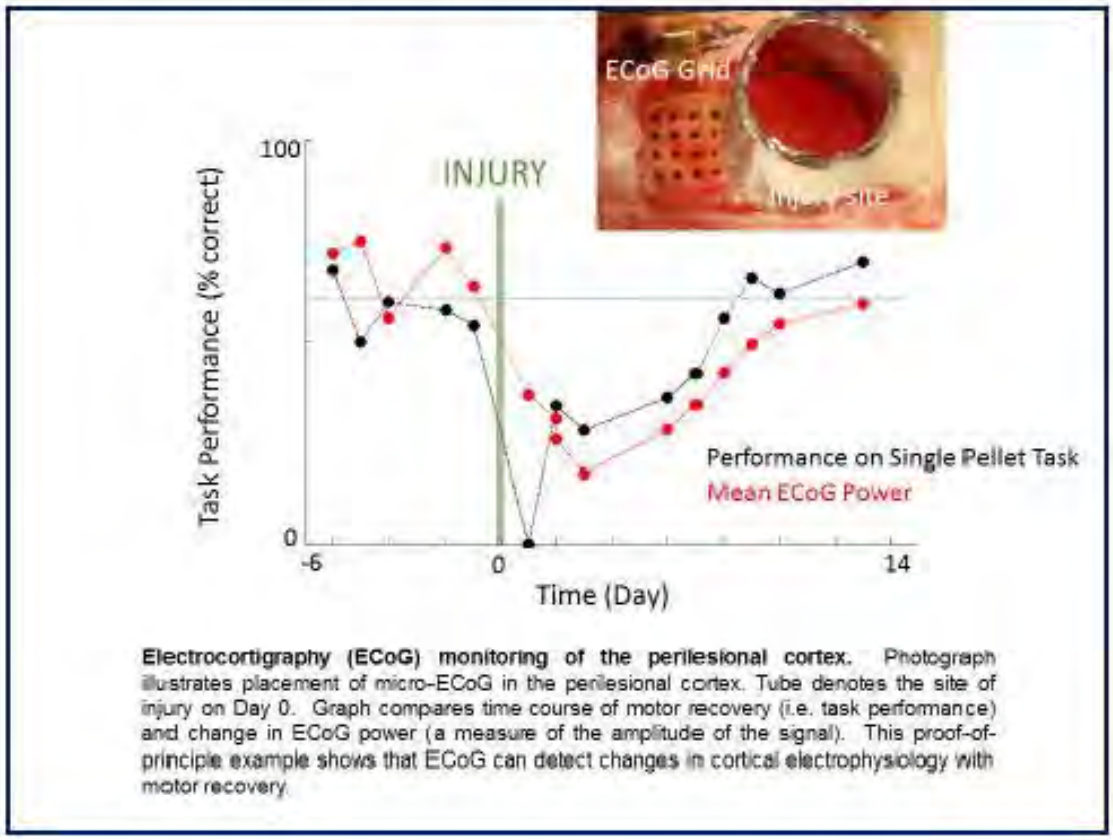
screen measure of cognitive abilities. These are pictured below, along with the other behavioral assessments we have put in place for rats and pigs. (Poster Presentation)



b) Electrocorticography. Although slated for year 2, we have finished preliminary studies using electrocorticograthy to record changes in brain network activity after injury. These have been able to yield stable traces over time and an excellent correlation with recovery of forelimb function. See 2 panels below:



Bihemispheric Electrocorticography (ECoG) Recordings. **A.** Customized recording array that can monitor the prefrontal/frontal/motor/pariental cortex in a rodent. **B.** Photograph of grid placement. **C.** Illustration of bilateral recordings of raw ECoG signals from a single anesthetized rodent.



Electrocortigraphy (ECoG) monitoring of the perilesional cortex. Photograph illustrates placement of micro-ECoG in the perilesional cortex. Tube denotes the site of injury on Day 0. Graph compares time course of motor recovery (i.e. task performance) and change in ECoG power (a measure of the amplitude of the signal). This proof-of-principle example shows that ECoG can detect changes in cortical electrophysiology with motor recovery.

What opportunities for training and professional development has the project provided?
The project has provided opportunities for training among the new staff, particularly w/ respect to the animal behavioral assessments. In addition, Ms. Bishop had the opportunity to present her work on this project at the California Neurotrauma symposium this year.

How were the results disseminated to communities of interest?
Results to date have been in the areas of model refinement and preliminary data collection. These have been disseminated as

a) an Oral presentation at the 2014 UCLA California Neurotrauma Symposium (09/14/2014); "True blast injury, fact or fiction?" and

b) a Poster presentation at the 2014 UCSF Biomedical Sciences graduate program retreat (09/10/2104) "Modeling Traumatic Brain Injury in Rats and Pigs"

What do you plan to do during the next reporting period to accomplish the goals?
Studies will proceed as described in the award proposal/SOW. In particular, we will proceed to studies involving behavioral outcome measures and the electrocorticography measures, and studies of ketogenic diet.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change. None

Actual or anticipated problems or delays and actions or plans to resolve them

a) A potential problem with the rat blast injuries is that we have found very little blast-induced injury when the head is fully immobilized. Ongoing studies aim to fully establish whether this is true even with repeated blast exposures. If so, we will conclude that blast exposure produces injury primarily by induction of head movement rather than the blast wave itself, and we will publish the data to support this. We will then proceed using the blast tube to induce head movement in association with accelerometers to document the extent of head movement.

b) Constructs for generating conditional CtBP2$^{-/-}$ mice are were sequenced and found to be correct, but 2 attempts at generating ES cells from these constructs failed. We are presently consulting locally to see if the new TALENS system will be more effective

Changes that had a significant impact on expenditures. None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents. None

Significant changes in use or care of human subjects. N/A

Significant changes in use or care of vertebrate animals. None

Significant changes in use of biohazards and/or select agents. N/A

6. PRODUCTS

Journal & book publications. None

Other publications, conference papers, and presentations.

a) Oral presentation at the 2014 UCLA California Neurotrauma Symposium (09/14/2014); "True blast injury, fact or fiction?"

b) Poster presentation at the 2014 UCSF Biomedical Sciences graduate program retreat (09/10/2104) "Modeling Traumatic Brain Injury in Rats and Pigs"

Website(s) or other Internet site(s). None.

Technologies or techniques. None.

Inventions, patent applications, and/or licenses. None

Other Products. None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Raymond A. Swanson MD
Project Role:	PI
Researcher Identifier	ORCID 0000-0002-3664-5359
Nearest person month worked	2.4
Contribution to project	Study design, personnel recruitment, compliance, data analysis
Funding support	This award

Name:	S. Scott Panter PhD
Project Role:	Faculty
Researcher Identifier	

Nearest person month worked	2.0
Contribution to project	Supervision of all studies done with pigs
Funding support	This award, Dept. of Veterans Affairs

Name:	Karunesh Ganguly, MD, PhD
Project Role:	Faculty
Researcher Identifier	
Nearest person month worked	1.5
Contribution to project	Mouse electrocorticography studies
Funding support	Dept. Veterans Affairs

Name:	Valerie Coppes
Project Role:	Large animal surgery technician
Researcher Identifier	
Nearest person month worked	3.0
Contribution to project	Conducted pig TBI and histology
Funding support	Dept. Veterans Affairs

Name:	David Kapfhamer, PhD
Project Role:	Research Scientist
Researcher Identifier	
Nearest person month worked	8.0
Contribution to project	Rat histology and behavioral assessments
Funding support	This award

Name:	Karen Hamel
Project Role:	Large animal surgery technician
Researcher Identifier	
Nearest person month worked	6
Contribution to project	Pig TBI, post-op monitoring, and histology
Funding support	This award

Name:	Robin Bishop, MS
Project Role:	Technician / Lab supervisor
Researcher Identifier	
Nearest person month worked	9.0

Contribution to project	Purchasing, stocking, coordinates studies, assists in behavioral assessments, conducts rat blast injury experiments.
Funding support	This award

Change in active other support of the PD/PIs or senior /key personnel
Nothing to Report

What other organizations were involved as partners?
None

8. SPECIAL REPORTING REQUIREMENTS
Not applicable

9. APPENDICES
None attached